CLAIMS:

1. An algorithm for determining neuronal structure by analyzing a microscopy image, said algorithm comprising:

a processing module for processing the image and extracting neuronal structures therefrom based on geometrical features of the neuronal structures; and

an analyzing module for analyzing the extracted neuronal structures to determine at least one characteristic thereof.

2. The algorithm according to Claim 1, wherein the image is selected from the group consisting of static image and time-series images.

3. The algorithm according to Claim 1, wherein the processing module performs a deconvolution process to extract the neuronal structures.

4. The algorithm according to Claim 3, wherein the extracted neuronal structures include a plurality of dendrites which are identified via their respective backbones.

5. The algorithm according to Claim 4, wherein the processing module detects from the plurality of dendrites a plurality of spines as geometric protrusions relative to the backbones.

6. The algorithm according to Claim 5, wherein the processing module subjects each geometric protrusion to a protrusion criterion to distinguish geometric protrusions associated with the plurality of spines from geometric protrusions not associated with the plurality of spines.

7. The algorithm according to Claim 6, wherein the processing module correlates each detached spine of the plurality of spines to its respective dendrite of the plurality of dendrites.

8. The algorithm according to Claim 5, wherein the analyzing module analyzes each of the plurality of spines to determine the at least one characteristic thereof.

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9.   The algorithm according to Claim 8, wherein the at least one characteristic thereof is selected from the group consisting of spine length, spine density and spine volume.

5   10.   The algorithm according to Claim 9, wherein the spine length for a spine detached from its respective dendrite is determined by the distance from a recorded dendrite surface volume element corresponding to the respective dendrite to a furthest spine volume element corresponding to the detached spine.

10   11.   The algorithm according to Claim 9, wherein the spine length for a spine fully or partially attached to its respective dendrite is determined by the distance from the center of mass corresponding to base boundary points associated with the fully or partially attached spine to a furthest spine volume element corresponding to the fully or partially attached spine.

15   12.   The algorithm according to Claim 9, wherein the spine density is computed as the number of spines per unit length of dendritic backbone.

13.   The algorithm according to Claim 9, wherein the spine volume is computed by multiplying the ratio of maximum spine intensity to maximum dendrite intensity by focal

20   volume.

14.   The algorithm according to Claim 5, wherein the analyzing module classifies each of the plurality of spines according to shape.

25   15.   The algorithm according to Claim 14, wherein each of the plurality of spines is classified in one of the following classifications: stubby, thin and mushroom.

16.   The algorithm according to Claim 14, wherein the analyzing module determines the shape of each of the plurality of spines based on spine length, spine head diameter and spine

30   neck diameter.

17.   The algorithm according to Claim 16, wherein a spine is classified as a thin spine if the spine length is greater than the neck diameter; a spine is classified as a stubby spine

if the neck diameter is approximately equal to the spine length; and a spine is classified as a mushroom spine if the spine length does not exceed neck diameter by more than a factor of 5 and the head diameter is greater than the neck diameter.

5   18.   A method for determining the effect of a substance on a neuron comprising:

subjecting the neuron to the substance;

imaging the neuron to generate at least one image;

subjecting the at least one image to an algorithm which contains (i) a processing module for processing the image and extracting neuronal structures therefrom based on

10   geometrical features of the neuronal structures and (ii) an analyzing module for analyzing the extracted neuronal structures to determine at least one characteristic thereof; and

comparing the at least one characteristic to a corresponding at least one characteristic of a control neuron.

15   19.   A method for determining the effect of a substance on a neuron according to claim 18, wherein subjecting the neuron to the substance involves entry of the substance into the neuron.

20.   A method for determining the effect of a substance on a neuron according to

20   claim 19, wherein the entry is accomplished by a transfection technique selected from the groups consisting of diffusion, electroporation, viral transfer, lipid mediated transfer, calcium phosphate precipitation, direct injection and biollistic transfer.

21.   A method for determining the effect of a substance on a neuron according to

25   claim 18, wherein the image is generated by laser scanning microscopy.

22.   A method for determining the effect of a substance on a neuron according to claim 21, wherein the laser scanning microscopy is selected from the group consisting of 2-photon exitation laser scanning microscopy and confocal laser scanning microscopy.

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23.   A method for determining the effect of a substance on a neuron according to claim 18, wherein the neuron is contained in a brain slice.

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24. A method for determining the effect of a substance on a neuron according to claim 18, wherein the image is selected from the group consisting of static image and time-series images.

5 25. A method for determining the effect of a substance on a neuron according to Claim 18, wherein the processing module performs a deconvolution process to extract the neuronal structures.

26. A method for determining the effect of a substance on a neuron according to
10 Claim 25, wherein the extracted neuronal structures include a plurality of dendrites which are identified via their respective backbones.

27. A method for determining the effect of a substance on a neuron according to Claim 26, wherein the processing module detects from the plurality of dendrites a plurality of
15 spines as geometric protrusions relative to the backbones.

28. A method for determining the effect of a substance on a neuron according to Claim 27, wherein the processing module subjects each geometric protrusion to a protrusion criterion to distinguish geometric protrusions associated with the plurality of spines from
20 geometric protrusions not associated with the plurality of spines.

29. A method for determining the effect of a substance on a neuron according to Claim 28, wherein the processing module correlates each detached spine of the plurality of spines to its respective dendrite of the plurality of dendrites.
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30. A method for determining the effect of a substance on a neuron according to Claim 27, wherein the analyzing module analyzes each of the plurality of spines to determine the at least one characteristic thereof.

30 31. A method for determining the effect of a substance on a neuron according to Claim 30, wherein the at least one characteristic thereof is selected from the group consisting of spine length, spine density and spine volume.

32.   A method for determining the effect of a substance on a neuron according to Claim 31, wherein the spine length for a spine detached from its respective dendrite is determined by the distance from a recorded dendrite surface volume element corresponding to the respective dendrite to a furthest spine volume element corresponding to the detached spine.

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33.   A method for determining the effect of a substance on a neuron according to Claim 31, wherein the spine length for a spine fully or partially attached to its respective dendrite is determined by the distance from the center of mass corresponding to base boundary points associated with the fully or partially attached spine to a furthest spine volume element corresponding to the fully or partially attached spine.

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34.   A method for determining the effect of a substance on a neuron according to Claim 31, wherein the spine density is computed as the number of spines per unit length of dendritic backbone.

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35.   A method for determining the effect of a substance on a neuron according to Claim 31, wherein the spine volume is computed by multiplying the ratio of maximum spine intensity to maximum dendrite intensity by focal volume.

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36.   A method for determining the effect of a substance on a neuron according to Claim 27, wherein the analyzing module classifies each of the plurality of spines according to shape.

37.   A method for determining the effect of a substance on a neuron according to Claim 36, wherein each of the plurality of spines is classified in one of the following classifications: stubby, thin and mushroom.

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38.   A method for determining the effect of a substance on a neuron according to Claim 36, wherein the analyzing module determines the shape of each of the plurality of spines based on spine length, spine head diameter and spine neck diameter.

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39.   A method for determining the effect of a substance on a neuron according to Claim 38, wherein a spine is classified as a thin spine if the spine length is greater than the neck

diameter; a spine is classified as a stubby spine if the neck diameter is approximately equal to the spine length; and a spine is classified as a mushroom spine if the spine length does not exceed neck diameter by more than a factor of 5 and the head diameter is greater than the neck diameter.

40.   A method for determining the effect of a substance on a neuron according to Claim 18, wherein the substance is selected from the group consisting of nucleic acid, protein, peptide, carbohydrate, lipid, metal, radiation, temperature, pH, drug, toxin, dye, virus, vitamin and mineral.

41.   A method for determining the effect of a substance on a neuron according to Claim 18, wherein subjecting the neuron to the substance includes exposure of the neuron to at least two dyes such that one dye illuminates the structure of a dendrite and a second dye illuminates distribution of a target molecule in the neuron.

42.   A method for determining the effect of a substance on a neuron according to Claim 41, wherein the second dye is a fusion protein comprising a fluorescent protein linked to a target protein of interest.

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